NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
Division of Extramural Research and Training
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Concept Clearance
for
Metabolic Profiling: Application to Toxicology and Risk Reduction

## Introduction

Metabolites are the end products of cellular processes and their levels are reflective of the systems level response of biological systems. Metabolic profiling, also called *metabonomics* or *metabolomics*, is a high throughput approach to measuring and interpreting complex, time-related metabolic parameters in biosamples such as urine, blood, cells and tissues. This initiative proposes a new approach for environmental health research using metabolic profiling for identifying and validating predictive markers of environmental exposure, toxicity and disease and for understanding the time course of toxicological response in relation to genetic susceptibility, age and environmental exposures.

## Background

There have been remarkable advances in the development of high throughput technologies for metabolic profiling. Technologies built on nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and chemical separation have made it possible to produce biochemical fingerprints of metabolites from complex biological mixtures such as biofluids and tissue extracts. Changes in fingerprint profiles can be used to characterize the effects of toxic insult or genetic manipulation in *in vivo* systems. Metabolic spectra are information-rich and computational tools, pattern recognition methods, and expert modeling systems are developing concurrently with technology advancements to maximize information recovery (1,2).

Metabolic profiling has widespread application in basic and applied research because it is nonselective and requires little sample preparation or derivitization. Many of the technologies for metabolic profiling have been developed by industry for chemical toxicity screening, drug discovery and genetic linkage studies. Metabolic profiling of urine or blood has been used to identify dose- and time-dependent biochemical profiles, site of toxic action, mechanism of toxicity, onset, duration and dose-response relationships in animals exposed to a variety of kidney and liver toxins including hydrazine, cadmium, mercury, and acetaminophen (3-9). In these studies, metabolic profiles of response were related to toxicity as determined using histopathology and clinical chemistry. The technology is highly sensitive because characteristic profiles of response can be defined for individual strains of animals, polymorphic individuals, and individuals exposed to chemical doses that did not induce toxicity based on histopathology or clinical chemistry (2, 6, 10). This is a great improvement over histopathology as a means of toxicological evaluation because once the metabolic pattern recognition spectra have been generated based on histopathology and clinical chemistry future studies require only collection of

urine from the experimental animals. Thus, studies following the time course and progression of toxicological events can be pursued without the need for sacrificing animals at each time point (6).

In the clinical arena, metabolic profiling has proven to be a valuable tool for diagnosing the severity and pathological condition of cardiovascular, renal and neurodegenerative diseases in animals and humans (10-14). The technique has also been used to produce metabolic markers for disease progression and identify metabolic pathways that are perturbed in dystrophic tissue from the *mdx* mouse model of Duchenne muscular dystrophy (10). Thus, metabolic profiling may be of great biological significance in studying genetic differences, including those due to specific intervention such as genetic knock out models. The technology may also be useful for validating animal models of human disease processes and defining predictive profiles for the time course of toxicological response. The search for clinical biomarkers of target organ toxicity and progression of toxicity in humans is often hindered by variation in factors such as genetic composition, diet, age and idiosyncratic responses to drugs. Metabolic profiling offers a nonselective approach for determining target organ toxicity and classifying effects of multiple organ toxicities based on readily obtainable samples. The technique provides a framework for reducing risk through the development of targeted prevention strategies.

The NIEHS has significant interest in assessing the usefulness of metabolic profiling as a tool for basic environmental science research. Congressional testimony on behalf of the NIEHS highlighted the importance of this emerging technology for toxicogenomics and systems biology research. Metabolic profiling was also featured at the Division of Extramural Research and Training Science Retreat in 2000 as a tool for advancing basic research aimed at elucidating mechanisms of action and surrogate markers of environmental exposure and disease. An international conference hosted by the NIEHS on *Metabolic Profiling: Application to Toxicology and Risk Reduction* on May 14-15, 2003 defined the state of the science for metabolic profiling and highlighted recent studies where metabolic profiling has been used to identify predictive markers of exposure, toxicity and disease in experimental animals and humans. Several speakers emphasized the promise of metabolic profiling for personalizing the assessment of environmental exposure and disease risk and elucidating systems responses to environmental agents. Understanding and quantitating the effect of environmental agents on the metabolome provides fingerprints of past and current exposure to environmental agents and early markers of impending toxicity or disease. The definition of such markers in animals, and eventually in humans, will improve the definition of exposure, toxicity and disease and may help identify genetic polymorphisms that define more susceptible individuals or populations.

**Research Goals and Scope**

This is a multi-phased initiative focused on the development and application of metabolic profiling technologies in basic and applied environmental science research.

Specific research foci include:

- ?? Studies to identify dose- and time-dependent biomarkers (predictive profiles) of exposure, toxicity and disease status and progression in animal models, including genetic

knock out models, and in specific human populations for which archived biosamples are available;

?? Studies to develop and validate animal models of human disease processes with emphasis on biological mechanisms and pathways, time course and genetic susceptibility in animal models and humans;

?? Development and application of bioinformatics, computational and modeling approaches for analysis of metabolic profiling data and its integration into models of systems levels responses to environmental exposures.

The initial phase of the initiative, to begin in 2004, will focus on identifying and validating predictive markers of exposure, toxicity and disease in order to establish proof of concept for metabolic profiling as a technology to further the understanding of risk and disease processes resulting from environmental exposures. One approach for implementing this phase of the initiative will be enhancing capacity at academic institutions which have demonstrated capabilities in chemical separation and identification technologies, such as liquid chromatography, gas chromatography, MS and NMR, which are amenable to metabolic profiling. Enhancing capabilities within these research institutions builds on existing expertise and instrumentation applicable to metabolic profiling and fosters research that is complimentary to existing programs in toxicology, genetics, toxicogenomics and proteomics. This phase of the initiative may also be coordinated with intramural efforts of the National Toxicology Program to identify predictive profiles of toxicity and disease using archived biological samples and information on dose- and time-dependent pathology and disease for environmental chemicals and drugs. A critical aspect of the initial and future phases of this initiative is the development and mining of metabolic profiling databases using novel bioinformatics, computational and modeling approaches.

While the focus of this initiative is on identifying predictive markers in animal models, retrospective studies using archived human biosamples are also of interest. Ideally, predictive markers could serve as indicators of early response to exposure and toxicity that can lead to development of appropriate prevention and intervention strategies that can be tested in these models. Future initiatives will focus on the development and validation of computational models of systems level responses to environmental agents and on establishing predictive profiles for human diseases processes and genetic susceptibility in clinical populations.

Ultimately, this initiative will foster the introduction of novel scientific ideas, methods, model systems, tools and technologies that have the potential to substantially advance basic and applied research in metabolic profiling and integrated systems sciences. This research initiative takes advantage of state-of-the-art technologies that can used to develop predictive markers of exposure, toxicity and disease in established animal models and to better understand mechanisms of toxicity and disease. Under this initiative, productive collaborations between academic institutions and private sector groups are encouraged as a means of promoting technology transfer and capacity building within academic institutions.

This initiative is timely as the new technologies of metabolic profiling complement existing and potential future NIEHS initiatives in genetic polymorphisms, toxicogenomics, proteomics and systems biology. Utilizing metabolic profiling techniques may, for the first time, offer real-time

evaluation of exposures and provide predictive markers for the time course and progression from exposure to disease. The data generated by this program may also be important for deciphering gene-environment interactions in disease and understanding the role of polymorphisms in susceptibility to disease.

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